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SHORT NOTE

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Inoculation of field-established mulberry and papaya with arbuscular mycorrhizal fungi and a mycorrhiza helper bacterium

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Abstract The effects of soil inoculation with arbuscular mycorrhizal (AM) fungi and a mycorrhiza helper bacterium (MHB) were investigated on mulberry and papaya plants already established in the field. Ten-year-old mulberry plants (var. M5) were inoculated with *Glomus fasciculatum* and 1.5-year-old papaya plants (var. Solo) were inoculated with a mixed culture of *G. mosseae* and *G. caledonium* with or without *Bacillus coagulans* at two levels of P fertilizer. Growth, P uptake, yield and AM colonization levels were monitored. Leaf yield in mulberry and fruit yield in papaya were minimal in uninoculated plants given 50% recommended P. However, crop yields of both mulberry and papaya inoculated with AM fungi alone or together with the bacterium and given 50% recommended P were statistically on a par with that of uninoculated plants given 100% recommended P. As inoculation of *B. coagulans* increased mycorrhiza levels in AM fungal-inoculated plants, this may be included in the class of MHB. Thus, mulberry and papaya already established in the field may respond to AM inoculation and MHB may increase symbiosis development by efficient AM fungi.

Keywords Mulberry · Papaya · Mycorrhiza helper bacterium · *Glomus*

Introduction

The use of arbuscular mycorrhizal (AM) fungi in enhancing plant growth and yield of many crops has gained momentum in recent years because of the higher cost and hazardous effects of heavy doses of chemical fertilizers. The extramatrical hyphae produced by AM fungi act as extensions of roots and increase the surface area of the root system, making it more efficient for absorption of water and diffusion-limited nutrients, this effect being more pronounced in P-deficient soils (Bagyaraj and Reddy 2000). Other beneficial effects are in the biological control of root pathogens, biological nitrogen fixation, hormone production and increased ability to withstand water stress. Most tropical soils are not only P-deficient but also P-fixing. The phosphate fertilizer application recommended for crops in the tropics is normally high and a large proportion remains unavailable to the crop plants to which it is applied. Crops generally take up less than 15–25% of fertilizer-applied phosphorus during the year of application (Brady 1990). Positive responses of crop plants to inoculation with efficient AM fungi under pot culture conditions has been well documented (Bagyaraj 1984; Jeffries 1987). It has been pointed out that AM inoculation could have a large economic impact on agriculture and horticulture if the beneficial effects of AM fungi observed under pot culture studies could be emulated under field conditions. Though benefits of AM fungi under field conditions have been reported for annuals and perennials inoculated in the nursery (Bagyaraj 1984; Chandrashekara et al. 1995), there is practically no information on the response of perennial crops already established in the field to inoculation with AM fungi. Hence, the present study examined the response of 10-year-old mulberry plants grown under rain-fed conditions in a garden, and 1.5-year-old papaya trees grown under drip irrigation under field conditions to AM inoculation either alone or together with a mycorrhiza helper bacterium (MHB) at two different levels of P fertilizer.

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Materials and methods

Experimental site and plantation

The experiments were conducted at the mulberry garden of the Department of Sericulture and at the papaya garden of the Department of Horticulture of the University of Agricultural

Sciences, Bangalore, India. The experiment on mulberry continued for 2 years and that on papaya for 1.5 years. The soil was an alfisol of a fine kaolinitic, isohyperthermic Typic Kanhaplustalfs type with a pH of 5.93, organic carbon of 0.56%, available nitrogen of 238 kg/ha, available phosphorus of 25 kg/ha (Bray's method) and available potassium of 30 kg/ha. Spacing of mulberry plants (*Morus alba* L. var. M5) was 75 cm between rows and between plants, and that of papaya plants (*Carica papaya* L. var. Solo) was 180 cm between rows and plants.

Experimental details

There were four treatments with 12 replications. All treatments received 100% recommended N and K. For mulberry, recommended N, P and K in this region are 100, 50, 50 kg/ha per year, respectively, nitrogen being applied in two doses. The first application of nitrogen was with the other fertilizers at the initiation of the experiment and the second dose was after the second harvest. NPK was applied in the form of urea, single super phosphate and murate of potash, respectively. Fertilizers were applied along the furrows on either side of the plant rows. Mulberry leaves were harvested five times per year at intervals of 70 days. For mulberry, there were two prunings per year, with top pruning at 70 cm from the soil level after second harvest and bottom pruning at 25 cm from the soil level after the fifth harvest. For papaya, NPK was applied at a rate of 250, 250 and 500 g/plant per year, respectively, the recommended level in the region for the variety used. Nitrogen was given in two doses, the first along with other fertilizers at the initiation of the experiment and the second at the time of flowering.

AM and *Bacillus coagulans* inoculum

AM inoculum *Glomus fasciculatum* (Thaxter Sensu Gerd.) Gerdemann and Trappe obtained form the University of California, Riverside, Calif., USA containing 2,083 infective propagules/g was used for mulberry. A mixed inoculum of *G. mosseae* (Nicol. and Gerde.) Gerdemann and Trappe, a local isolate containing 140 infective propagules/g, and *G. caledonium* (Nicol. and Gerd.) Gerdemann and Trappe obtained from the University of Western Australia, Nedlands, Australia containing 1,736 infective propagules/g was used for papaya. These AM fungi were chosen as they were effective fungi for the two perennials in earlier studies (Balakrishna Reddy et al. 1996; Umakanth and Bagyaraj 1998).The most probable number method with 10-fold dilution was used for estimating the number of infective propagules (Porter 1979). The AM fungi used were maintained at the Department of Microbiology, University of Agricultural Sciences, Bangalore, India in a sterilized sand: soil mixture (1:1 by volume) with Rhodes grass (*Chloris gayana* L.) as host. The inoculum containing the substrate with extramatrical hyphae, spores and infected root pieces of Rhodes grass was used at the rate of 100 g/plant.

The bacterium *B. coagulans* isolated in this laboratory and found to enhance colonization by AM fungi (Caroline and Bagyaraj 1995) was grown in 500-ml conical flasks containing nutrient broth for 48 h on a shaker. The cultures were then centrifuged at 5,500 rpm for 7 min. The supernatant was discarded and the pellet containing bacterial cells was suspended in 250 ml of 0.1 M $MgSO_4.7H_2O$ and adjusted to give 70% transmittance in a Spectronic 20 spectrophotometer (Milton Roy) at 520 nm. A sample was taken to determine the population of *B. coagulans* in serial dilutions on nutrient agar by the poured plate method. Cell suspensions containing 10^6 cfu/ml were added to each plant. Four holes were made around each plant near the root zone to a depth of about 12 cm. Aliquots (30 ml) of *B. coagulans* and 100 g of AM inocula were added per plant (7.5 ml of *B. coagulans* and 25 g of AM inocula per hole) 12 days after fertilizer application.

Treatments were: *T1* uninoculated plants given 100% recommended P, *T2* uninoculated plants given 50% recommended P, *T3* inoculated with AM fungi and given 50% recommended P, and *T4* inoculated with AM fungi plus *B. coagulans* and given 50% recommended P.

Plant growth, leaf yield, P concentration and AM root colonization

Five labelled plants from each treatment were exclusively used for biometric observations. For mulberry, height, number of shoots per plant, number of leaves per plant and leaf yield per plant of the selected plants were measured at 70-day intervals. The measurements in each year were averaged. The plant P concentration was determined by the vanadomolybdate method (Jackson 1973). Fine roots from a radial distance of 30 cm from stem base and up to 30 cm deep were collected at four points around the plant, which has an adventitious root system. The percentage AM root colonization in mulberry after the final harvest was determined by the gridline intersect method (Giovannetti and Mosse 1980) after staining the roots with acid fuchsin, as described by Kormanik and McGraw (1982).

In papaya, plant height and stem girth was recorded at 30 day intervals and the means of 12 observations for the first year and 6 observations for the second year were calculated. The means of 2 years are reported. Similarly, the number of flowers and fruits were recorded from their formation for a period of 1.5 years at monthly intervals. The roots were collected as for mulberry and the percentage AM root colonization in papaya, which has a tap root system, was determined as mentioned earlier.

Statistical analysis

The data obtained from the experiments were subjected to analysis of variance (ANOVA) using MSTAT software. All treatment means were tested for least significant difference at the 5% level of significance and the means were separated by Duncan's multiple range test (Little and Hills 1978).

Results and discussion

The present investigations on field-established rain-fed mulberry plants and drip-irrigated papaya plants were carried out in order to evaluate the possibility of reducing application of phosphate fertilizer through microbial inoculation. Inoculation of well-established 10-year-old mulberry plants and 1.5-year-old papaya plants with AM fungi significantly increased plant height, number of leaves and stem girth. Plant height and number of leaves per plant in mulberry, and plant height and stem girth in papaya were maximal when plants were inoculated with AM fungi and given 50% recommended P. Means of this treatment were not significantly different from those of plants inoculated with AM fungi plus *B. coagulans* given 50% recommended P or those of uninoculated plants given 100% P (Tables 1, 2). Uninoculated mulberry plants given 50% P had the lowest plant height and number of leaves. The number of shoots in mulberry did not vary significantly between treatments. Several workers have investigated the field response of annuals and nursery raised crops to AM fungal inoculation. Iqbal and Qureshi (1972) reported 85% increase in height of sunflower plants inoculated with AM fungi compared with uninoculated controls under field conditions. Similarly, AM fungal inoculation in the nursery or at the time of planting

Table 1 Effect of soil inoculation with *Glomus fasciculatum* and *Bacillus coagulans* at two P levels on plant height, number of shoots, number of leaves per plant, fresh leaf yield, leaf P concentration and % AM root colonization of mulberry established in the field for 10 years. Data are the means of results over 2 years. Means in each column followed by different letters are significantly different ($P \leq 0.05$) according to Duncan's multiple range test (*T1* uninoculated plants given 100% recommended P, *T2* uninoculated plants given 50% recommended P, *T3* inoculated with AM fungi and given 50% recommended P, and *T4* inoculated with AM fungi plus *B. coagulans* and given 50% recommended P)

Treatment	Plant height (cm)	No. of shoots	Number of leaves/plant	Fresh leaf yield (g/plant)	Fresh leaf yield (kg/ha)	Leaf P concentration (%)	AM colonization (%)
T1	132.85a	11.9a	244.7a	277.50a	4925.75a	0.24ab	27.25bc
T2	124.49b	11.4a	236.1b	259.99b	4607.16b	0.21bc	31.58b
T3	137.95a	12.5a	255.8a	281.63a	5018.60a	0.26a	39.25ab
T4	140.59a	12.4 a	258.2a	287.85a	5103.93a	0.25a	44.50a

Table 2 Effect of soil inoculation with *Glomus mosseae* + *Glomus caledonium* and *Bacillus coagulans* at two P levels on plant height and stem girth (means of 2 years), number of flowers per plant (n=13), fruit yield per plant (n=10–13) and % AM root colonization of papaya established in the field for 1.5 years. Means in each column followed by different letters are significantly different ($P \leq 0.05$) according to Duncan's multiple range test. Treatment abbreviations as in Table 1

Treatment	Plant heigh (cm)	Stem girth (cm)	Number of flowers/plant	Fruits/plant	Fruits harvested	Fruit weight (kg/plant) (%)	AM colonization
T1	213.43a	26.68a	18.98a	32.57a	15.01a	3.10a	22.25c
T2	201.62bc	25.35b	15.91b	26.25b	11.67b	2.10b	22.08c
T3	215.59a	27.82a	19.10a	33.11a	15.21a	3.25a	37.23b
T4	214.79a	28.42a	19.15a	33.59a	15.78a	3.56a	41.08a

resulting in improved plant height and growth under field conditions has been reported in crops such as maize (Powell and Bagyaraj 1984), mulberry (Katiyar et al. 1995), finger millet (Govinda Rao et al. 1993) and sunflower (Chandrashekara et al. 1995).

Leaf yield in mulberry, and number of flowers and fruit yield in papaya, followed a trend similar to plant height (Tables 1, 2). Mulberry plants inoculated with AM fungi alone or AM fungi plus *B. coagulans* given 50% P and uninoculated control plants given 100% P had a significantly higher leaf yield than uninoculated control plants given 50% P. This is important since fresh mulberry leaves are fed to silkworms. Similar observations were made on the number and weight of papaya fruits. Such positive relations between plant growth, biomass and yield and AM inoculation have been observed in annuals and nursery-raised crops under field conditions (Govinda Rao et al. 1993; Chandrashekara et al. 1995). Katiyar et al. (1995) obtained a maximum yield of 30–36 t/ha per year by pre-inoculating mulberry plants with *G. fasciculatum* after reducing P application by 75% under irrigated field conditions. Inoculation of *B. coagulans* together with AM fungi significantly increased neither the leaf yield of mulberry nor the fruit yield of papaya compared with AM fungal inoculation alone. Mulberry plants inoculated with AM fungus alone or AM fungus plus *B. coagulans* given 50% P had a significantly higher leaf P concentration than uninoculated plants given either 50% or 100% P. AM fungi allow the root system to exploit a greater volume of soil P by extending away from the roots and translocating P away from the root zone (Rhodes and Gerdemann 1975), by exploiting smaller soil pores not reached by the root hairs, and by adding surface area to the adsorptive system (O' Keffe and Sylvia 1990).

Mycorrhizal root colonization was lowest in uninoculated plants given 100% P and highest in plants inoculated with AM fungus plus *B. coagulans* and given 50% P, for both mulberry and papaya (Tables 1, 2). Some bacteria present in the mycorrhizosphere (like MHB) can modify the establishment of mycorrhizal symbiosis (Garbaye and Brown 1989). Increased AM colonization in the presence of *B. coagulans* classifies this bacterium as MHB. It has been suggested that MHB produce hydrolytic enzymes which dilate the cortical cells and provide wider intercellular spaces through which AM fungi penetrate and ramify more easily in the root system (Caroline and Bagyaraj 1995).

In conclusion, the results demonstrate that field-established mulberry plants and papaya trees respond to inoculation with efficient AM fungi. Furthermore, P fertilizer application can be reduced by 50% through AM inoculation without reducing yield. Inoculation of field-established plants with AM fungi may be of interest for improving yield of other perennial crops.

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